Title: Development of a biosensor for urea assay based on amidase inhibition, using an ion-selective electrode

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Abstract: A biosensor for urea has been developed based on the observation that urea is a powerful active-site inhibitor of amidase, which catalyzes the hydrolysis of amides such as acetamide to produce ammonia and the corresponding organic acid. Cell-free extract from Pseudomonas aeruginosa was the source of amidase (acylamide hydrolase, EC 3.5.1.4) which was immobilized on a polyethersulfone membrane in the presence of glutaraldehyde; anion-selective electrode for ammonium ions was used for biosensor development. Analysis of variance was used for optimization of the biosensorresponse and showed that 30 mu L of cell-free extract containing 7.47 mg protein mL(-1), 2 mu L of glutaraldehyde (5%, v/v) and 10 mu L of gelatin (15%, w/v) exhibited the highest response. Optimization of other parameters showed that pH 7.2 and 30 min incubation time were optimum for incubation ofmembranes in urea. The biosensor exhibited a linear response in the range of 4.0-10.0 mu M urea, a detection limit of 2.0 mu M for urea, a response timeof 20 s, a sensitivity of 58.245 % per mu M urea and a storage stability of over 4 months. It was successfully used for quantification of urea in samples such as wine and milk; recovery experiments were carried out which revealed an average substrate recovery of 94.9%. The urea analogs hydroxyurea, methylurea and thiourea inhibited amidase activity by about 90%, 10% and 0%, respectively, compared with urea inhibition.

Author Keywords: Ion-Selective Electrode; Aliphatic Amidase; Urea Biosensor

KeyWords Plus: Pseudomonas-Aeruginosa; Ethyl Carbamate; Kinetic-Properties; risk Assessment; Milk Urea; Enzyme; Organophosphorus; Cholinesterase; Pesticide; Nitrogen

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